in addition to its significantly higher S/B ratio. It requires minimal amount of organic-anion transporter inhibitor (such as probenecid) in its assay system. In conclusion, Cal-590 AM is a greatly improved red fluorescent indicator for measuring intracellular calcium change. The red-shifted wavelength makes Cal-590 AM a robust tool for evaluating GPCR and calcium channel targets, multiplexing analysis of cellular functions with a green fluorescent dye as well as screening agonists and antagonists with GFP cells.

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Coupling Interactions of the Dimeric SOAR Unit of STIM1 with Orai1 Channels

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The STIM-Orai activating region (SOAR) of STIM1 is the minimal sequence necessary for Orai1 channel activation. Our previous work revealed that replacement of Phe-394 in SOAR with the dimensionally similar but polar histidine head group prevents Orai1 binding and gating. In the current study, we constructed YFP-SOAR-SOAR concatemers with a 21 amino acid linker between the SOAR units which allowed folding into functional dimers. We introduced the F394H mutation (FH) into either the first (N-terminal) or second (C-terminal) SOAR unit within the dimeric concatemer constructs. We found that each of these single-mutated concatemers was able to both bind to Orai1 and gate the Orai1 channel normally, the same as the wild-type concatemer. In contrast, a concatemer in which both SOAR units contained the F394H mutation (YFP-SOAR^{FH}-SOAR^{FH}), was devoid of Orai1 binding and gating activity. To ensure that the single mutated concatemers were not forming inter-concatemer dimers, we equally co-expressed the CFP-SOAR^{WT}-SOAR^{WT} and YFP-SOAR^{FH}-SOAR^{FH} concatemeric constructs in the same cell. While the CFP-SOAR^{WT}-SOAR^{WT} bound to and activated Orai1, it did not pull the YFP-SOAR^{FH}-SOAR^{FH} with it. Thus, the latter remained in the cytosol in these cells. This result is in contrast to co-expression of the monomeric CFP-SOAR^{WT} with YFP-SOAR^{FH} constructs. In this case, while the YFP-SOAR^{FH} expressed alone does not bind to or activate Orai1, the CFP-SOAR^{WT} clearly dimerizes with YFP-SOAR^{FH}mutant and pulls it to the PM. This also proves that he F394H mutation does not prevent SOAR-SOAR dimer formation. Overall, the results reveal a surprising new facet of the STIM1-Orai1 coupling interaction. Thus, although the SOAR dimer is likely the functional Orai1-actiavting unit, only one of the SOAR units within the SOAR dimer needs to be able bind to and activate the Orai1 channel.

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Translocation between PI(4,5)P_2-Poor and PI(4,5)P_2-Rich Microdomains During Store Depletion Determines STIM1 Conformation and Gating of Orai1

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Receptor-stimulated Ca^{2+} influx, a critical component of the Ca^{2+} signal, is mediated in part by Orai1, which is activated by STIM1 in response to Ca^{2+} release from the ER. Orai1 then undergoes slow Ca^{2+}-dependent inactivation (SCDI) that is mediated by binding of SARAF to STIM1. We use SCDI by SARAF as reporter of the conformation and microdomain localization of the Orai1-STIM1 complex. Interaction of STIM1 with the C terminus of Orai1 and the STIM1 K-domain are required for interaction of SARAF with STIM1 and Orai1 SCDI. Interaction of SARAF with STIM1 required the presence of STIM1-Orai1 in a PM/ER microdomain that is tethered by E-Syt1, stabilized by Septin4 and enriched in PI(4,5)P_2. Notably, selective targeting of STIM1 to PI(4,5)P_2-rich or to PI(4,5)P_2-poor microdomains revealed that SCDI by SARAF is observed only when the STIM1-Orai1 complex is within the PI(4,5)P_2-rich microdomain. Most notably, measuring the dynamics of STIM1-Orai1 complex localization in live cells using PI(4,5)P_2-rich or PI(4,5)P_2-poor microdomain probes revealed that store depletion is followed by transient STIM1-Orai1 complex formation in the PI(4,5)P_2-poor microdomain where the channel is fully active, which then translocates to the PI(4,5)P_2-rich domain to recruit SARAF and initiates SCDI. These findings reveal the role of the PM/ER tethers in the regulation of Orai1 function and Ca^{2+} influx and describe a new mode of regulation by PI(4,5)P_2 involving translocation between PI(4,5)P_2 microdomains, rather than by PI(4,5)P_2 synthesis and breakdown.

Cardiac Smooth and Skeletal Muscle Electrophysiology I

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Differential Effects of Antiarrhythmic Drugs Vernakalant and Flecainide on Human Two-Pore-Domain K^+ Channels

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Atrial fibrillation (AF) contributes significantly to cardiovascular morbidity and mortality. The growing epidemic is associated with cardiac repolarization abnormalities and requires the development of more effective antiarrhythmic strategies. Cardiac two-pore-domain K^+ channels repolarize action potentials and represent potential targets for AF therapy. However, electropharmacology of K2P channels remains to be investigated in detail. This study was designed to elucidate human K2P channel regulation by antiarrhythmic drugs vernakalant and flecainde.
Two-electrode voltage clamp and whole-cell patch clamp electrophysiology was used to record K2P currents from Xenopus oocytes and Chinese hamster ovary (CHO) cells. The class III antiarrhythmic compound vernakalant activated K2P17.1 currents in oocytes and in mammalian cells (EC50,CHO = 40 μM) in frequency-dependent fashion. K2P17.1 open rectification characteristics and current-voltage relationships were not affected by vernakalant. In contrast to K2P17.1, vernakalant reduced K2P4.1 and K2P10.1 currents, in line with K2P2.1 blockade reported earlier. The class I drug flecainide did not significantly modulate K2P currents.
In conclusion, vernakalant activates K2P17.1 background potassium channels. Pharmacologic K2P channel activation may be employed for personalized rhythm control in patients with AF-associated reduction of K^+ channel function.

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Classifying the Electrophysiological Effects of Chronotropic Drugs on Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes using Voltage Sensitive Dyes and Supervised Machine Learning

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The emergence of human induced pluripotent stem (hiPS) cell technology has expanded the possibilities for sourcing human cardiomyocytes (hiPS-CMs). Novel microscopy and analysis methods serve to accelerate development and validation of *in vitro* hiPS-CM models for drug screening. Voltage sensitive dyes (VSD) allow non-invasive, non-destructive, and longitudinal assessment of hiPS-CM electrophysiology at the sub-cellular membrane scale. In this study, we successfully use 2-photon microscopy to capture VSD signal at the cellular membrane scale generated from actively beating hiPS-CMs exposed to the chronotropic drugs, propranolol (10^{-5} M) and isoproterenol (10^{-7} M). We use SimFCS software, developed at the Laboratory for Fluorescence Dynamics at the University of California, Irvine, to remove motion artifact and assess the resultant signal over time. We are able to generate a waveform of VSD fluorescence that is representative of the changing membrane potential (i.e. the depolarization of an action potential). A number of characteristics of these waveforms are defined (upslope, maximum height, plateau height, downslope, peak width, and beat rate), compared across treatments, and shown to be significantly different between treatments. A supervised machine learning algorithm is then trained, validated, and the algorithm accuracy quantified using these data along with their known drug treatments. The algorithm that results can be used to predict which drugs hiPS-CMs have been exposed to given only their respective VSD waveforms. This study tests the hypothesis that VSDs may be used in conjunction with supervised learning to train an algorithm that is capable of automatically and accurately assessing, classifying, and predicting the membrane depolarization effects of chronotropic drugs.

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Sparfloxacin, a Fluoroquinolone Antibiotic, Slows Inactivation of L-Type Ca^{2+} Current in Neonatal Rat Ventricular Myocytes

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Introduction: The proarrhythmic effects of quinolone antibiotics used clinically have been assessed by measuring the I_{Kr} antagonist potency. However, the gaps between clinically reported proarrhythmic effects and I_{Kr} antagonist